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Langerhans Cells Come in Waves

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It is unclear how the Langerhans cell (LC) network is maintained in adult epidermis. In this issue of *Immunity*, Seré et al. (2012) show that LCs are replenished in two waves. Monocyte-derived, short-lived LCs come first. A second wave follows, and these LCs of nonmonocytic origin are long-lived.

Langerhans cells (LCs) are dendritic cells of epithelia. The best-studied example is LCs of the epidermis (Romani et al., 2010). From the early experiments, it has been appreciated that there is something special about the life cycle of epidermal LCs. When human skin was transplanted onto nude mice, human LCs within the graft persisted for a remarkably long period of several months (Krueger et al., 1983). Studies from others have corroborated these observations (Merad et al., 2002). It was established that (1) LCs populate the epidermis early in ontogeny and (2) they are very long-lived. The LC network is established within a few days after birth by an initial burst of proliferation as demonstrated in murine epidermis (Chorro et al., 2009). In human skin, precursors of LC are present in the embryonic skin, and they gradually acquire LC-specific markers during development of the fetus (Schuster et al., 2009). With regard to longevity, LCs essentially persist for the entire lifetime of a mouse, provided the epidermis is not disturbed by inflammation. Even in human hand transplants, the LCs of the hand donor remained in place for almost a decade (Kanitakis et al., 2011).

From this body of evidence, one could infer that LCs in the steady-state adult epidermis are a largely independent cell population that renews itself and does not need fresh supplies from progenitor cells in bone marrow or the dermis. In contrast, another nonembryonic LC precursor cell has been reported (Ginhoux et al., 2006). During skin inflammation induced by UV-C irradiation, which largely depletes resident LCs from the epidermis, LCs are subsequently replenished by immigrating monocytes that are characterized by the expression of the Gr-1 marker. Once in the epidermis, monocytes acquire langerin (also known as CD207) expression.

In spite of the seminal findings mentioned above, the homeostasis of LCs in the adult epidermis is still not entirely clear. Can monocytic LC precursors fully make up for the inflammation-induced lack of LCs? Do these "monocyte-derived LCs" in the epidermis live equally long (i.e., virtually life-long) as the "normal" LCs that populated the epidermis around birth? How exactly is the epidermis reconstituted with LCs after an inflammation-induced efflux of LCs? In this issue of *Immunity*, Seré et al. (2012) made use of mice with deleted transcription factor Id2, which strikingly lack all LCs (Hacker et al., 2003) and langerin$^+$ dermal dendritic cells. Thus, the mice are devoid of any langerin-expressing cells in the skin. When wild-type (WT) bone marrow was injected into these adult Id2-deficient mice, a network of LCs established over time, suggesting that the repopulation in the steady-state occurred by bone marrow-derived precursors that acquire MHC-II first, followed by langerin expression. This is similar in the newborn. Shortly after birth and settlement in the epidermis, both markers sequentially come up on LCs (reviewed in Romani et al., 2010). The time course was protracted in the chimeric mice (few weeks after bone marrow transfer) as compared with newborn (few days after birth). The authors then undertook a set of experiments to dissect repopulation during skin inflammation, and to this end, they resorted to the UV model originally described by Merad et al., (2002). UV-C irradiation led to inflammation in the skin and to the loss of LCs from the epidermis within 1 week. At the same time, both epidermis and dermis were strongly infiltrated by Gr-1$^+$ monocytic cells. Within the following 4 weeks, the epidermis of WT mice was repopulated by dendritic cells, which acquired MHC-II and high langerin expression in a sequential fashion, as it may have seemed at first glance. Id2-deficient mice,

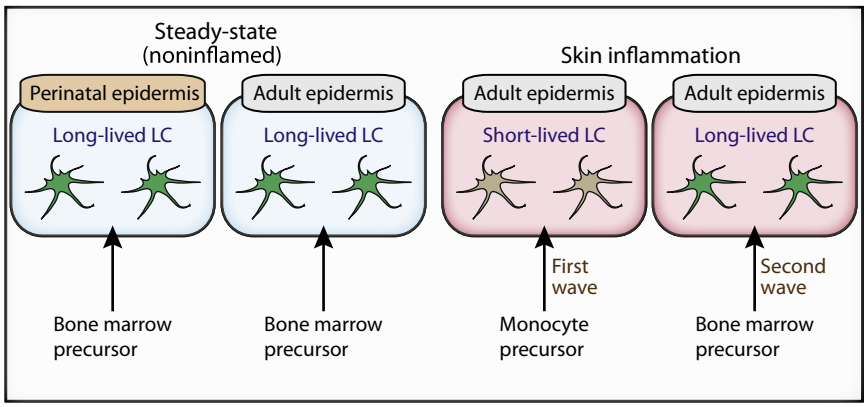

Figure 1. Langerhans Cells Populate the Epidermis in Different Ways
In newborn, one wave of bone marrow precursors enters the epidermis within a few days and proliferates. In adult Id2-deficient mice or diphtheria-toxin depleted mice, LC precursors immigrate gradually into the epidermis. It is unclear to what degree they proliferate there. In response to inflammation-induced LC depletion of the epidermis, LCs return in two waves. The first wave is derived from Gr-1$^+$ monocytes. These LCs stay only transiently. However, the Id2-dependent, monocyte-independent wave of LCs is destined to stay within the epidermis for a long time. Short-lived LCs express markedly less langerin than long-lived LCs.

however, gave a different answer. Only cells that expressed low amounts of langerin entered the epidermis; langerin-high cells did not appear. A time-course after UV treatment showed that in WT mice the initial subset of langerin-low LCs disappeared over the weeks, whereas langerin-high LCs increased proportionally and were still readily detectable 12 weeks after UV. They may thus be considered as long-lived. This led the authors to the conclusion that in response to inflammation there are two types of LC that come into the epidermis in two waves (Figure 1).

This notion was corroborated in the subsequent experiments. Langerin$^+$ short-term LCs did indeed stem from Gr1$^+$ cells as demonstrated by the adoptive transfer of Gr1$^+$ monocytes purified from blood. In vivo clodronate-tagged monocytes migrated into the epidermis and expressed langerin. In addition, short- and long-term LCs were phenotypically different, short-term LCs resembling monocytes (e.g., high CD11b) and long term LCs being similar to resident, "normal" LCs (e.g., high CD205, CD24, and EpCam). TGF-β target genes like Smad7 and Id2 were upregulated in long-term LCs, similar to steady-state LCs.

Repopulation of the epidermis after specific depletion of LCs by diphtheria toxin, a commonly used model for depletion of langerin-expressing cells (Romani et al., 2010), was recently studied (Nagao et al., 2012). In this noninflammatory setting, LC repopulation occurred in distinct groups of MHC-II$^+$langerin$^+$ LCs. In addition, a small subset of MHC-II$^+$ but langerin$^-$ cells, named "preLC" by the authors, appeared in the epidermis. This subset looked phenotypically similiar to the short-term LCs (higher CD11b; less EpCAM and CD205) described by Seré et al. However, the cells did not express Gr1 and are thus unlikely to be directly derived from inflammatory monocytes. Nevertheless, "preLCs" also show a myelo-monocytic phenotype as defined by Lysozyme M (LysM). During inflammation induced by a contact allergen, repopulation was accelerated and this subset of MHC-II$^+$ langerin$^-$ cells differentiated into langerin$^+$ LCs. Thus, both Seré et al. and Nagao et al. show a short-term repopulation of the epidermis with LCs derived from myelo-monocytic precursors. Seré et al. (2012) showed that these "short-term" LCs disappear and are replaced by a second wave of "long-term" LCs that are not derived from monocytes. By contrast, Nagao et al. (2012) infer that their LysM$^+$ "preLCs" may differentiate into "true LCs." Indeed, even 9 weeks after the inflammatory stimulus, all LCs in the epidermis still express the LysM marker. The authors concede, however, that these long-lived LCs are only a subpopulation of LCs. Their study did not address the major population of non-monocytic long-lived LCs. In fact, when they depleted their myelo-monocytic LysM$^+$ "preLCs" (by using LysM-DTA mice, where cells die as soon as they begin to express LysM), they still observed repopulation of the epidermis by langerin$^+$ LCs. They deduce from this "the proposal of at least two sources of LCs in the adult mice." Seré et al. (2012) provide here a conclusive and detailed description of this "proposal."

Birbeck granules have been regarded as an ultrastructural hallmark for LCs. The presence (or not) of these granules is particularly intriguing because the expression of langerin in the "short-term LCs" is quite low. Is the combination of MHCII expression and low langerin expression sufficient to define these cells as LCs? We believe it is, but it would still be interesting and assuring to know whether the expression of such low amounts of langerin indeed correlates with the ultrastructural presence of Birbeck granules. Unfortunately, this task is extremely difficult. Tiny numbers of highly purified subset of cells would have to be processed for electron microscopy (EM). Because Birbeck granules in mouse LCs are not as abundant as in human LCs, large numbers of cells would have to be sampled in order to obtain a reliable judgment. In situ immuno-EM would also be extremely challenging due to the low frequency of these cells. It should be mentioned that it is for the same reason that we also do not know whether langerin$^+$ dermal dendritic cells possess Birbeck granules.

Besides their relevance as LC identifiers, Birbeck granules are of eminent importance for antigen handling. It has been shown that they are involved in antigen incorporation, presentation of glycolipid antigens, and neutralization of HIV and perhaps other viruses. This leads to the following question: why do we actually need these "short-term LCs"? Are they simply placeholders for the slower arriving "long-term" LCs, that we are tempted to call "real LCs"? Do they take over the role of the "long-term LCs" as a first line of defense against invasion of pathogens? Thus, functional analysis of short- and long-term LC would be interesting. Phenotypical differences are suggestive. The distribution of MHC-class II

and langerin molecules varied markedly between short- and long-term LCs, perhaps pointing to different modes of antigen handling and presentation. Differential expression of the C-type lectins DEC-205 (CD205), DC-SIGN (CD209), and even langerin may be indicative for different spectra of microorganisms that are dealt with by the two types of LCs.

Do monocyte-derived LCs, which enter the epidermis in response to a preceding, inflammation-induced loss of LCs, stay there for long-term and behave like "real" LCs? Seré et al. clearly show that this is not the case. In the adult epidermis, the persisting LC network is composed of LCs derived from an Id2-dependent precursor in the bone marrow, like in newborn. Monocyte-derived LCs settle in the epidermis only transiently. The presented work not only solves problems but also identifies questions to be tackled. Do long-term LCs also contribute to homeostasis in the absence of an initial depletion of LCs? In the Seré et al. study, UV irradiation was the trigger for the subsequent immigration of both short-term and long-term LCs. Does low level immigration of long-term LCs help to maintain the steady-state density of the LC network, in addition to self-renewal by local proliferation? Clearly, the work of Seré et al. forms a good base to build upon further research into this important cell type of the skin immune system.

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CEACAM1-S: The Virtues of Alternative Splicing in Gut Immunity

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Immunoglobulin A (IgA) is the main intestinal antibody. In this issue of *Immunity*, Chen et al. (2012) show that intestinal T cells enhance protective IgA responses by expressing a short isoform of the CEACAM1 protein.

The intestine is home to trillions of commensal microbes that confer many metabolic capabilities to the host and fill a niche that would otherwise be accessible to pathogens (Sansonetti, 2004). In spite of expressing a wealth of microbial sensors, intestinal tissues peacefully coexist with commensals without inducing inflammation. This homeostatic balance involves a continuous dialog between microbes and intestinal immune cells, which leads to the generation of a dynamic state of hyporesponsiveness against commensals and active readiness against pathogens. A key mediator of intestinal homeostasis is immunoglobulin A (IgA), an antibody isotype produced by B cells through class switching (Cerutti, 2008). IgA confers a noninflammatory tone to the intestine by shaping the composition of commensals (Wei et al., 2011). IgA production and release onto the mucosal surface require a close interplay of B cells with T follicular helper (Tfh) cells, T regulatory (Treg) cells, dendritic cells, follicular dendritic cells, and epithelial cells (Cerutti, 2008; Suzuki et al., 2010; Tsuji et al., 2009). In spite of recent advances, the mechanisms regulating intestinal IgA responses remain unclear.

In this issue of *Immunity*, Chen et al. (2012) show that intestinal T cells control commensal bacteria and protect against pathogens by inducing IgA through a pathway involving preferential expression of a short isoform of carcinoembryonic